

# **Risk assessment for the deployment of a Costa Rican transgenic rice variety under tropical conditions**

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COSTA RICA**

# Costa Rica

Extension: **51,000 Km<sup>2</sup>**

Population: **4 millions**

Economy based on: **ecotourism, agriculture and high technology industry.**

**38%** of the territory **is protected** in governmental and private conservation areas.

**5%** of world biodiversity, including more than **11.000** plant species

**National Biosafety Committee** since **1990**.

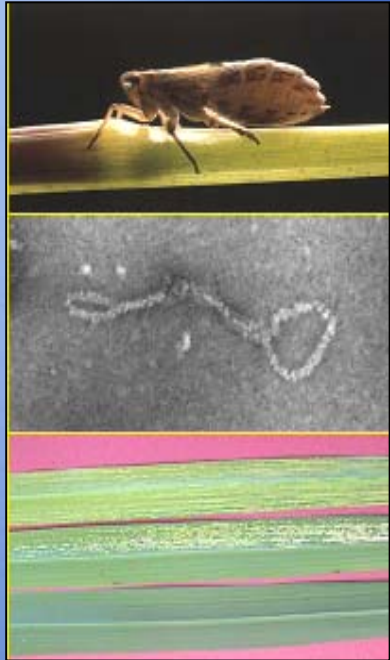
National policies are:

**Sustainable** use of biodiversity and biotechnology.





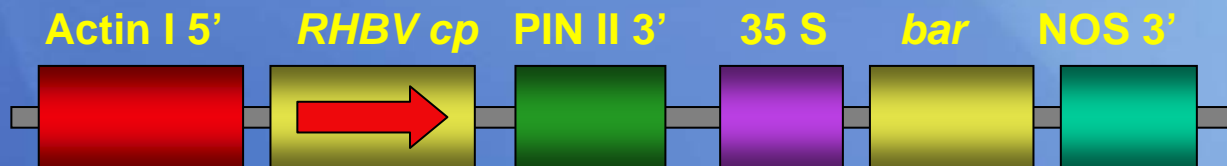
# Genetic engineering of a Costa Rican *indica* rice variety by biolistics



Sequences of rice hoja blanca virus (RHBV) nucleoprotein



*Bar* gene to confer resistance to herbicide PPT



# Field evaluation of transgenic lines

## Four field trials

- **Morphological evaluation of lines**
- **Resistance to the herbicide PPT and to RHBV**
- **Selection of lines with best agronomic traits**

## Confined conditions

- **Field trials in non rice growing areas**
- **Covered with bird-proof net**
- **Vegetative barriers surrounding field plot**
- **Destruction of remanents**
- **NBC supervision**





# Main objective

To study gene flow from *Oryza sativa* transgenic lines to related **wild rice species** and **weedy rice**

Transgenic rice lines



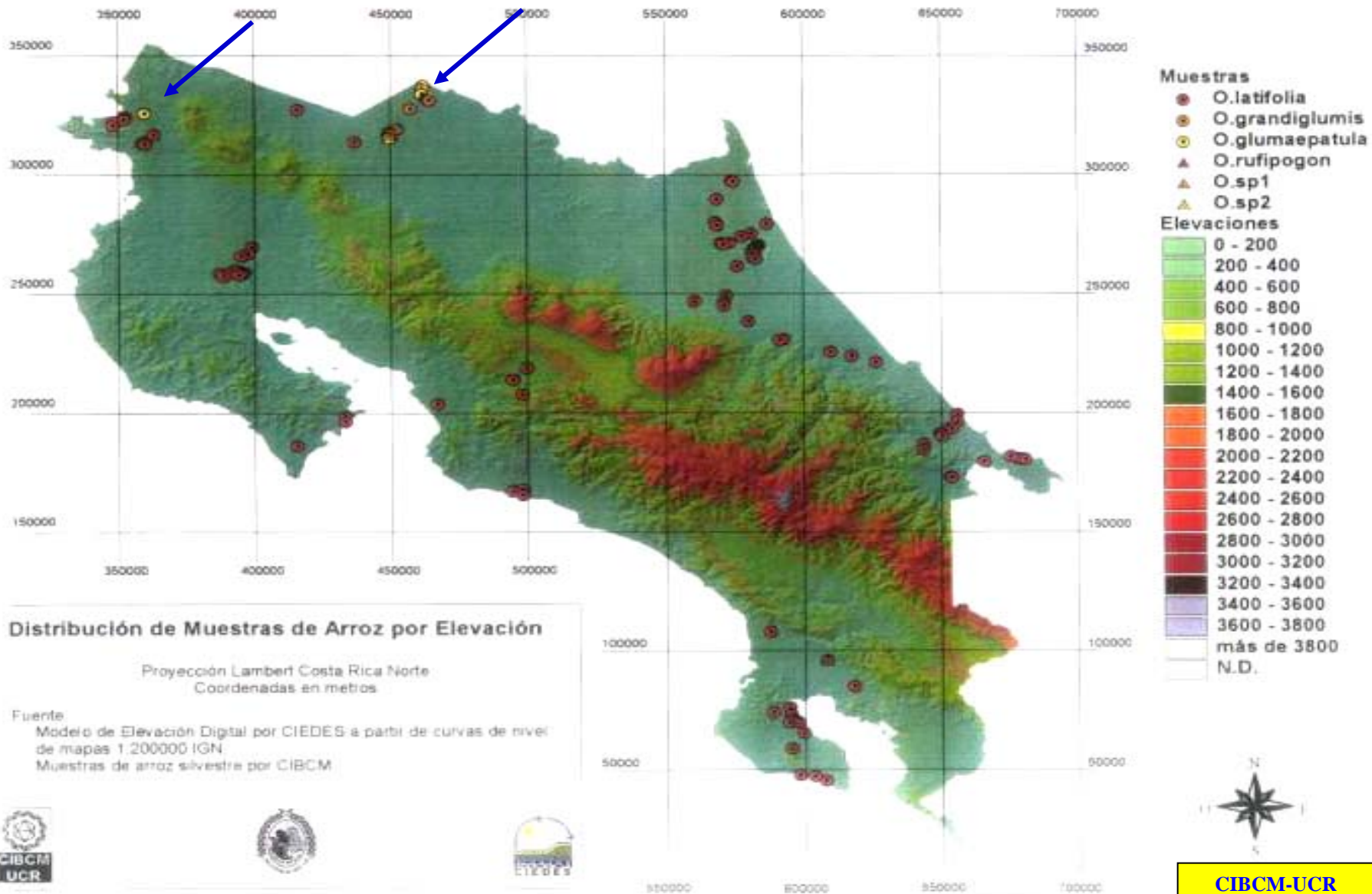
*Oryza glumaepatula*



Weedy rice



# Distribution of wild *Oryza* populations in Costa Rica

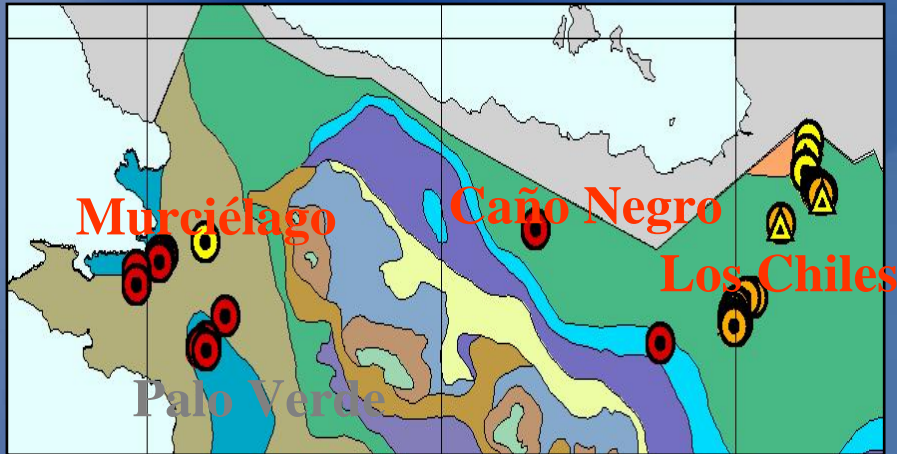




# Genome type of three native wild *Oryza* species of Costa Rica:

- *O. latifolia*: CCDD genome
- *O. grandiglumis*: CCDD genome
- *O. glumaepatula*: **AA** genome
  
- *O. sativa*: **AA** genome

# Reproductive biology of *O. glumaepatula*



- The Medio Queso Wetland is home for millions of *O. glumaepatula* plants.
- Synchronize flowering in different localities.
- Flowering period in November at the end of the rainy season.
- Rice commercial fields are grown in close proximity to *O. glumaepatula* (flowering period: August).



To study the reproductive biology of *O. glumaepatula* and its potential hybridization with *O. sativa*

To study the reproductive strategies of *O. glumaepatula*.

To determine the compatibility of *O. sativa* and *O. glumaepatula*.

To confirm the hybrid nature of the F<sub>1</sub> plants.

To determine seed set of the hybrids.

# Methodologies

## Reproductive biology

Selection of 19  
progeny groups.



PCR

labelled-SSR



Genetic  
analyses with  
sequencer



Comparison of  
genetic profiles

## Genetic compatibility

Flower emasculation



Hand made crosses  
(*O. glumaepatula*  
x *O. sativa*)



Seed set  
evaluation



## Hybrid seed evaluation

*In vitro* germination  
of the F<sub>1</sub> seeds



Morphological  
analyses



PCR

labelled-SSR



Genetic  
analyses with  
sequencer



# *O. glumaepatula* progeny studies using labelled-microsatellites

Typical segregation of self-pollinated plants

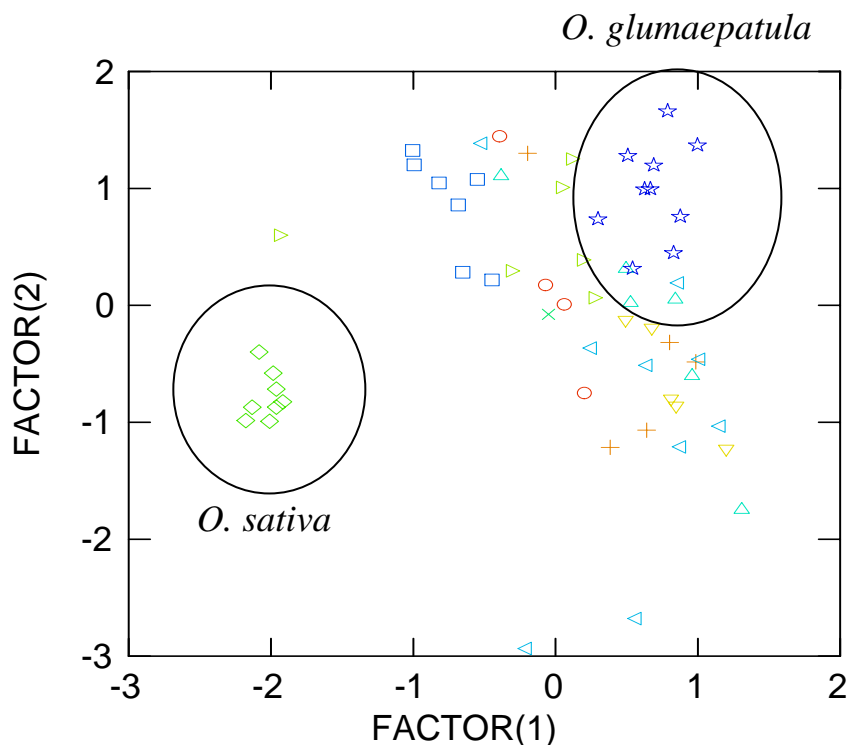
Sample	Family	RM5	RM13	RM17	RM22	RM255	RM38
I-1-2M	Female	ab	aa	aa	ab	aa	aa
I-1-2A	Progeny	ab	aa	aa	ab	aa	aa
I-1-2B	Progeny	bb	aa	aa	ab	aa	aa
I-1-2C	Progeny	ab	aa	aa	bb	aa	aa
I-1-2D	Progeny	ab	aa	aa	ab	aa	aa
I-1-2F	Progeny	aa	aa	aa	aa	aa	aa
I-1-2G	Progeny	ab	aa	aa	ab	aa	aa

Registration of outcrossing events

Family	RM5	RM13	RM17	RM22	RM255
Female	aa	aa	aa	aa	aa
Progeny	aa	aa	aa	aa	aa
Progeny	aa	aa	aa	aa	aa
Progeny	ab	aa	aa	aa	ab
Progeny	aa	aa	aa	aa	aa
Progeny	aa	aa	aa	aa	aa
Progeny	ab	aa	aa	aa	aa

# Evaluation of the hybrids using 12 morphological traits

## Discriminant analysis



Factor 1	Factor 2
Plant size	Ligule length
Color of the culm base	Flag length
Color of the stigma	Awn color

Variety

*O. glumaepatula*

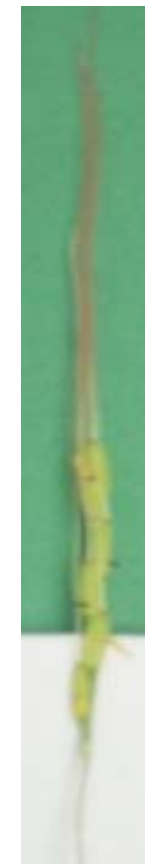
Hybrid



Awnless



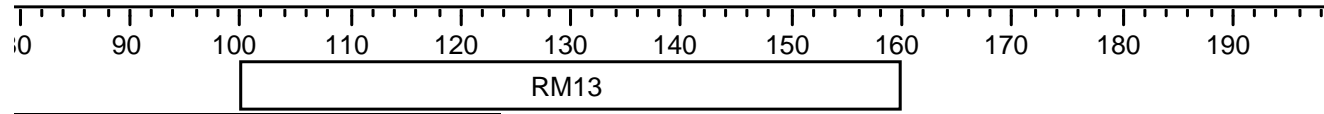
Straw  
awns



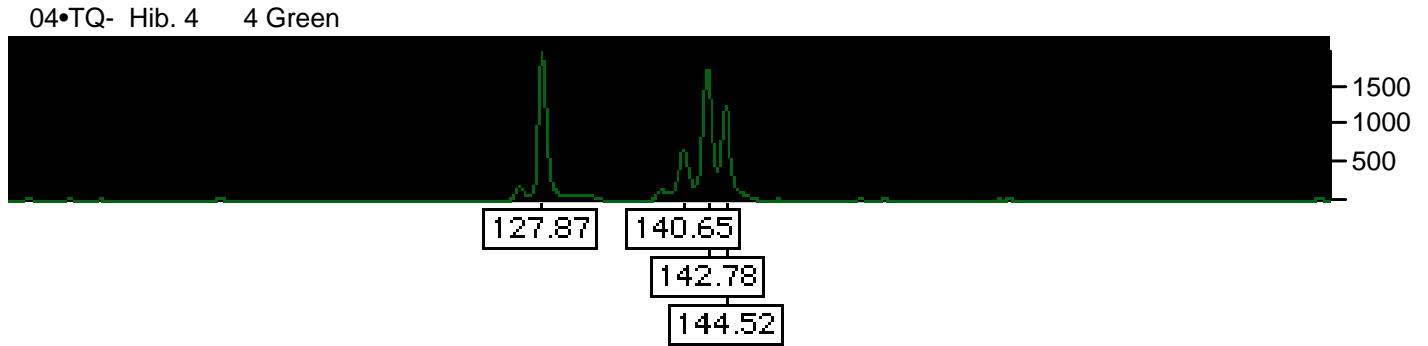
Pink awns



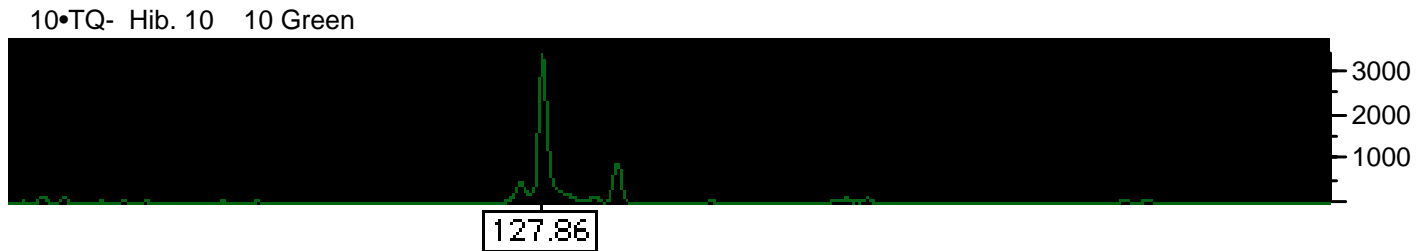
# Confirmation of the hybrid nature of the plants using fluorescent-labelled microsatellites



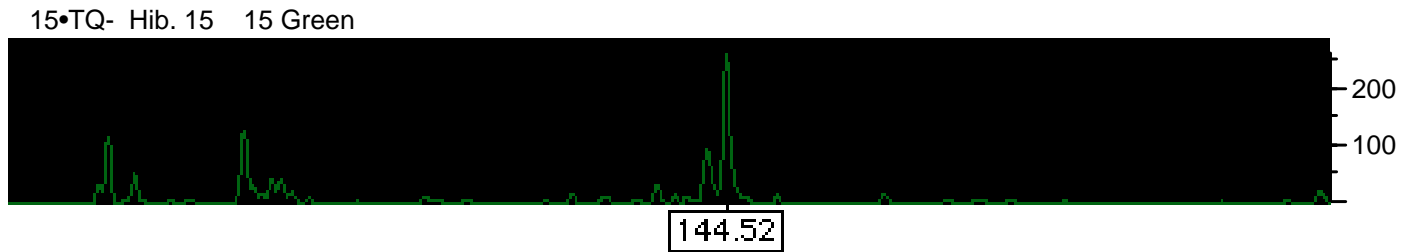
Hybrid 2211-6



*O. glumaepatula*



CR-4338



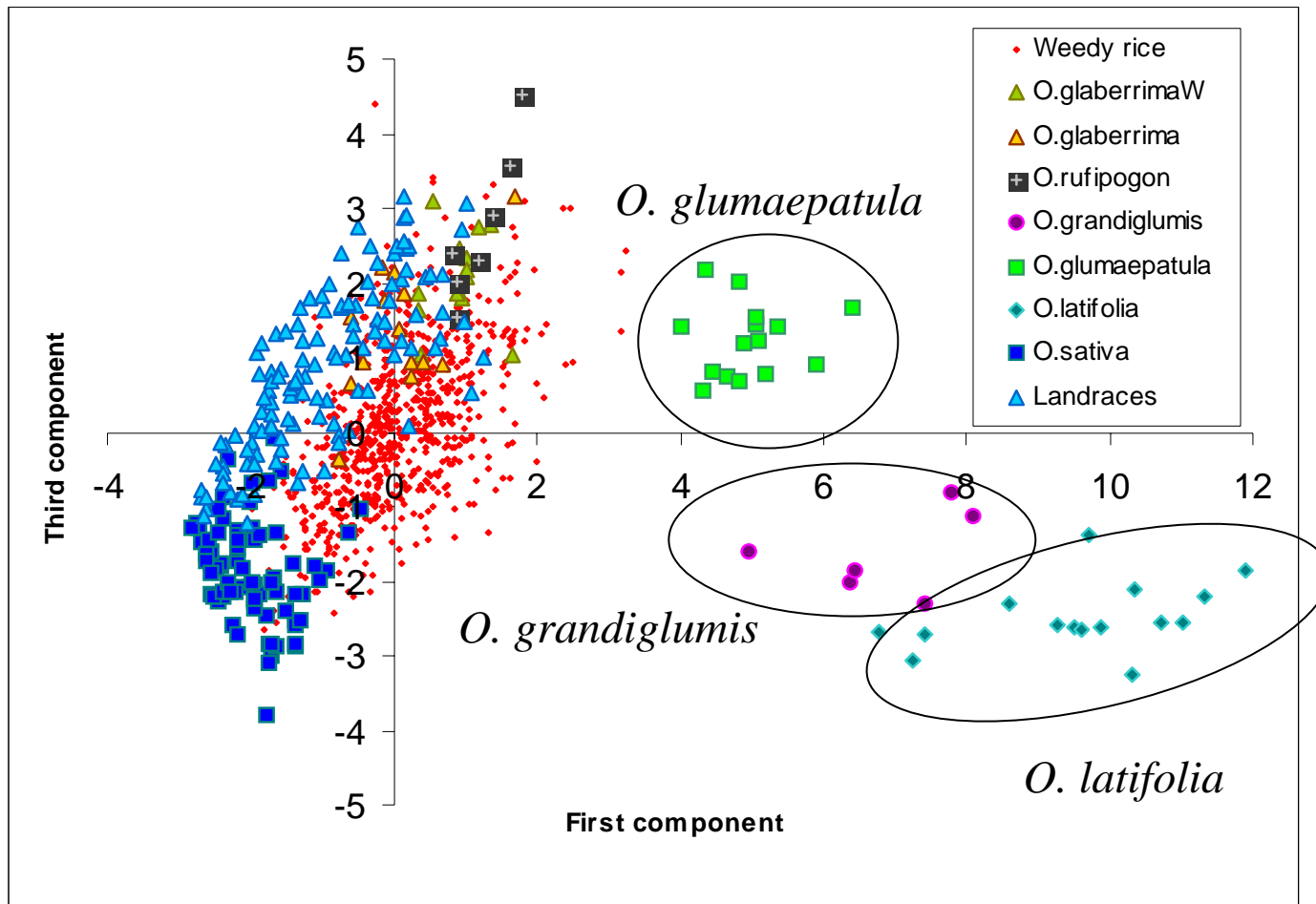
RM13 Genotyper Electrophenogram

# Conclusions

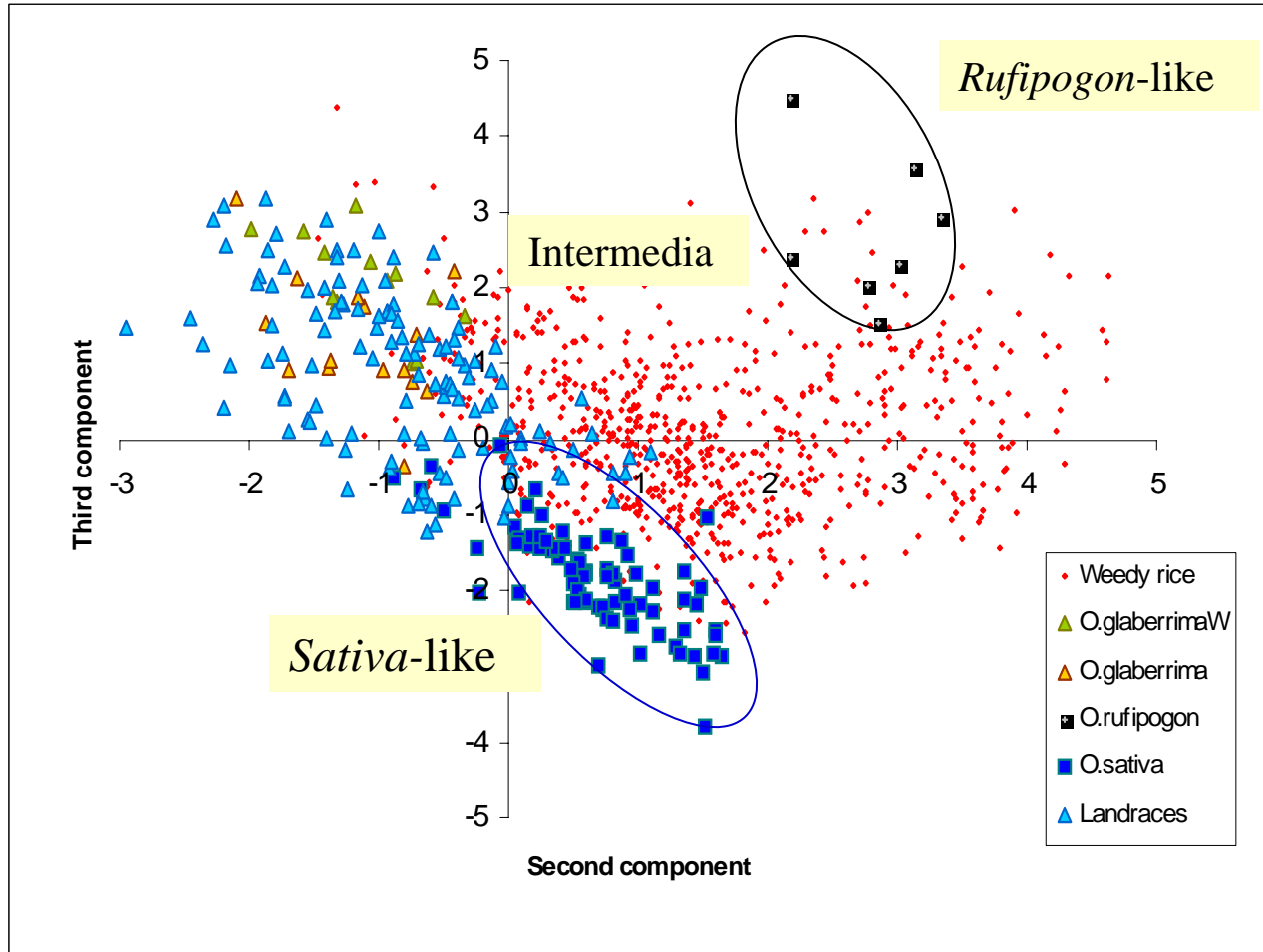
- *O. glumaepatula* is **autogamous** with few outcrossing events.
- Hybrids are **sterile**.
- **Lack of flowering overlap** between *O. glumaepatula* and *O. sativa*.
- The previous results suggest low probabilities of gene flow between these species. This information has important implications in terms of **biosafety**.



# Morphologic characterization of Costa Rican weedy rice complex



# Morphologic characterization of Costa Rican weedy rice complex



20  
morphotypes  
using seed  
traits

3 main  
groups:  
*sativa*-like  
*intermedia*  
*rufipogon*-like



# Evaluation of gene flow from transgenic rice variety to weedy rice

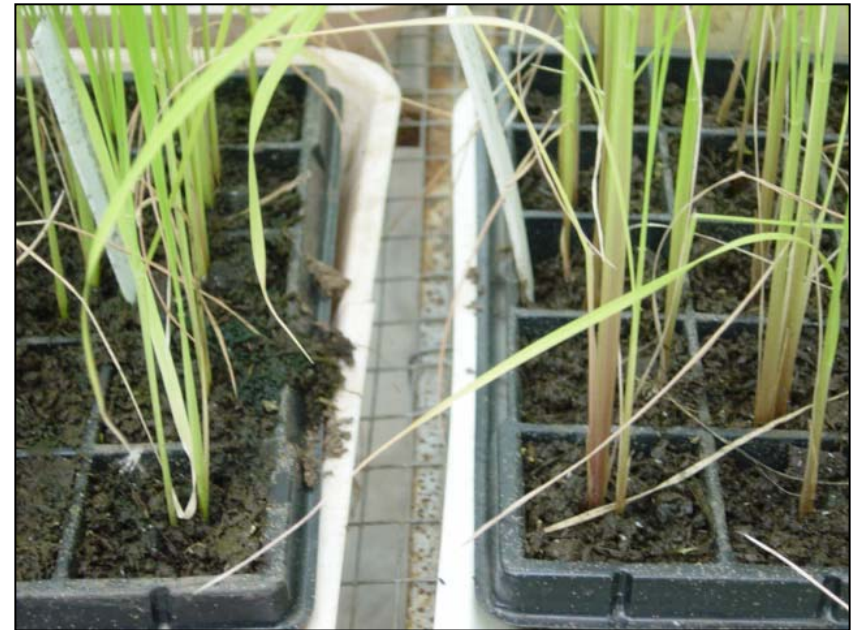
To assess gene flow from *O. sativa* to selected weedy rice morphotypes using two selection markers:

- The purple color of the culm of the variety Setesa-9.
- The resistance to ammonium glufosinate (PPT) of a transgenic rice line.



# Evaluation of gene flow from Setesa-9 to weedy rice

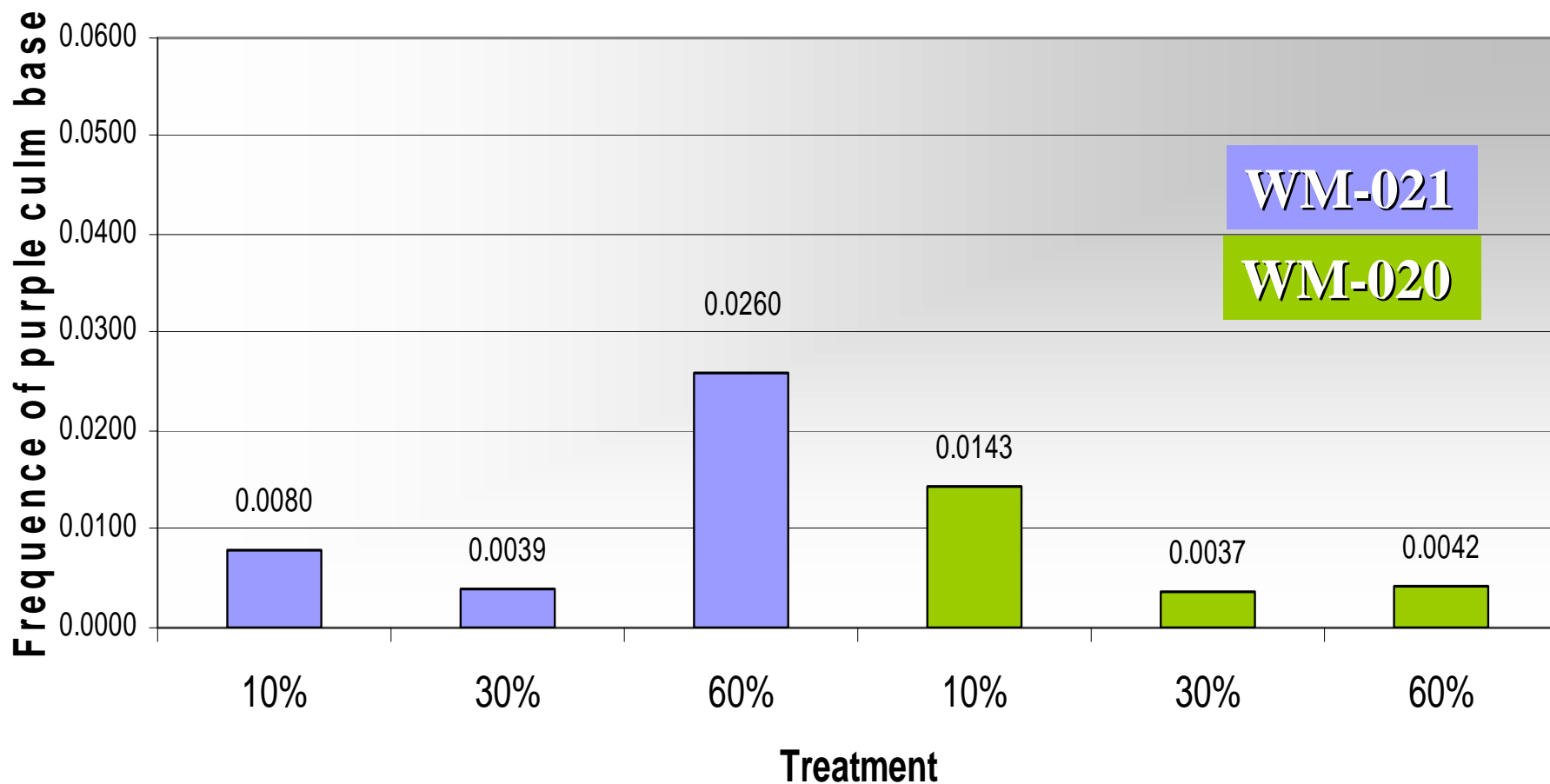
- Variety: **Setesa-9**
- Weedy rice morphotypes: **WM-020** and **WM-021**
- Plots of 2.25 m<sup>2</sup>
- Weedy rice infestation levels: **10%**, **30%** and **60%**
- Three replicates
  
- Seed harvest
- Evaluate F1 (purple culm base)
- Confirmation of putative hybrids using SSR.



WM-020

Setesa -9

# Frequency of putative hybrids Setesa 9 -weedy rice morphotypes



N = 55460









# Evaluation of gene flow from transgenic rice line to weedy morphotypes

- **Transgenic** rice line
- **Six** weedy morphotypes.
- **Plots with infestation levels:**  
10%, 15% and 30%
- 2 replicates 36 plots
- Harvest by rows 2 times/week
- PPT resistance **in  $F_1$** .
- Hybrid confirmation by **PCR**.





# Characteristics of weedy rice morphotypes used in the gene flow studies

	Weedy morphotype	Abundance in fields	Days to reach 50% anthesis	Morphological clasification
	<b>WM-020</b>	<b>19.6%</b>	<b>118</b>	<b>Sativa-like</b>
	WM-023	11.8%	101	Intermedia
	WM-073	2-5%	111	Intermedia
	WM-120	2-5%	115	Sativa-like
	WM-121	2-5%	106	Intermedia
	<b>WM-329</b>	<b>7.1%</b>	<b>101</b>	<b>Rufipogon-like</b>



# Hybrid identification (preliminary results)

- 4200-8000 weedy rice seeds per plot + 800 control seeds.
- Seed emergence evaluated 15 days after seeding (DAS)
- Herbicide dose:  
1<sup>st</sup> application 450g a.i./ha 30 DAS  
2<sup>ed</sup> application 450g a.i./ha 40 DAS
- Evaluation 10 days after 2<sup>nd</sup> application





# Results



- Weedy rice reproductive cycle

Morphotype	Clasification	50% anthesis	Flow ering overlap
WM-120	sativa-like	86	11
WM-020	sativa-like	90	14
WM-023	Intermedia	86	11
WM-121	Intermedia	86	14
WM-073	Intermedia	<b>98</b>	11
WM-329	rufipogon-like	90	7
AS10-7-14	transgenic line	<b>98</b>	

## Frequency of weedy rice herbicide resistant plants

Morphotype	Infestation level	Number herbicide resistant plants	Plants evaluated	Fequency	Percentage
WM-020	10%	7	5350	0.00131	0.13
WM-020	15%	8	7410	0.00108	0.11
WM-020	30%	34	8105	0.00419	0.42
WM-023	10%	4	4293	0.00093	0.09
WM-023	15%	12	7438	0.00161	0.16
WM-023	30%	4	8000	0.00050	0.05
WM-073	10%	5	7282	0.00069	0.07
WM-073	15%	5	7826	0.00064	0.06
WM-073	30%	3	8321	0.00036	0.04
WM-120	10%	36	6844	0.00526	0.53
WM-120 (sl)	15%	54	7443	0.00726	0.73
WM-120	30%	58	8032	0.00722	0.72
WM-121	10%	6	7318	0.00082	0.08
WM-121	15%	3	5436	0.00055	0.06
WM-121	30%	13	9550	0.00136	0.14
WM-329	10%	8	5967	0.00134	0.13
WM-329	15%	7	7148	0.00098	0.1
WM-329 (rl)	30%	2	6815	0.00029	0.03
<b>Total</b>		<b>269</b>	<b>128578</b>	<b>0.00209</b>	<b>0.21</b>



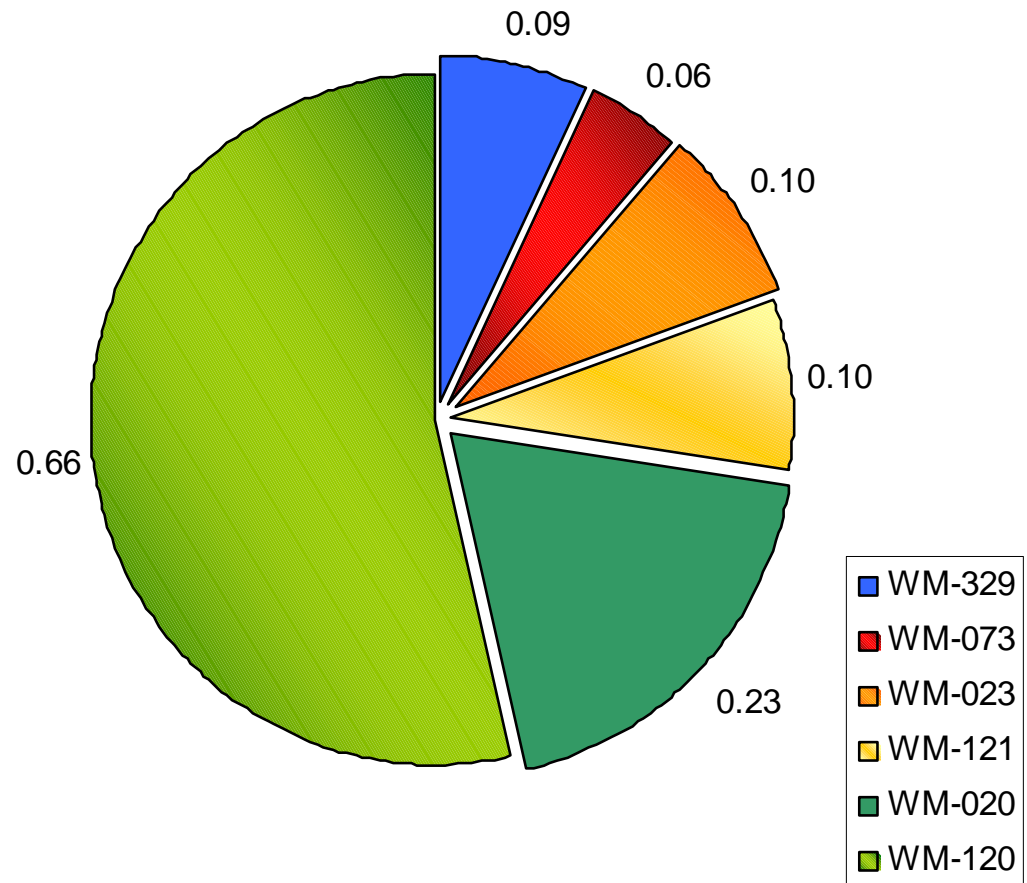
Hybrid *bar*<sup>+</sup>



## Differences between morphotypes

Morphotype	Frequency
WM-120	0.0131 <sup>a</sup>
WM-020	0.0030 <sup>b</sup>
WM-023	0.0010 <sup>c</sup>
WM-121	0.0010 <sup>c</sup>
WM-329	0.0008 <sup>c</sup>
WM-073	0.0006 <sup>c</sup>

Medias comparison GLZ Proc  
GENMOD SAS



## Herbicide resistant plants per weedy rice morphotype



# Conclusions

## Gene flow weedy rice-Setesa-9

- Hybridization percentage were higher than those reported for *O. sativa*.
- The purple color could be inherited in a more complex way and have different degrees of penetrance.
- No differences were observed in hybrid frequency according to infestation levels.

## Gene flow weedy rice-transgenic rice

- Hibridization between weedy rice and transgenic rice varied between 0.03% and 0.73%. Similar to other reports (Chen *et al.*, 2004; Messeguer *et al.*, 2004).
- Herbicide resistant plants were more common in sativa-like than in intermedia and rufipogon-like morphotypes.
- No differences were observed in hybrid frequency according to infestation levels.

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